Does CMV Trigger CD4+CD28null T cell Expansion in the Context of MS?

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INTRODUCTION

Cytotoxic CD4+CD28null T cells arise during chronic activation of the immune system, in a subset of healthy controls (HC) and patients with multiple sclerosis (MS), a disabling autoimmune disease of the central nervous system. CD4+CD28null T cells may contribute to MS, since they have cytotoxic properties (perforin, granzyme B, NK-cell receptors), accumulate in active lesions and at least a subpopulation is autoreactive in nature [BROUX (2012, 2015)]. So far, the cause of the expansion of CD4+CD28null T cells and their contribution to MS disease pathology is poorly investigated. There is mounting evidence that the expansion occurs after infection with cytomegalovirus (CMV), a double stranded virus of the β-herpes family [BROUX (2012), VANHEUSDEN (2015)]. Furthermore, CMV could possibly worsen the MS diseases course, although the opposite has also been stated [VANHEUSDEN (2015)].

OBJECTIVES

We hypothesize that CMV triggers the expansion of CD4+CD28null T cells and thereby aggravate MS disease.

Our main research questions are:
1) Does CMV infection trigger the expansion of CD4+CD28null T cells?
2) Do CMV and CD4+CD28null T cells play a role in MS?

METHODS

An association study between CMV serology and CD4+CD28null T cells in MS patients and HC was performed. In an in vitro assay, PBMCs from HC were stimulated with CMVpp65 and/or IL-2 to mimic chronic CMV stimulation. In vivo, mice were infected with CMV and at different time points, their splenocytes were isolated, stimulated with a CMV peptide mix and analyzed via flow cytometry. SNPs flanking MICB (rs2523651) and TLR2 (rs5743708), related to CMV infection, were investigated via Taqman and associated with CD4+CD28null T cell percentages and CMV serology. Furthermore, CD4+CD28null T cells were studied in experimental autoimmune encephalomyelitis (EAE, MS model) and the role of CMV and these cells in CMV infected EAE mice was examined.
RESULTS

The association study between CMV serology and CD4+CD28null T cells in MS patients and HC showed that CD4+CD28null T cells are increased in CMV+ donors (p<0.0001) and CMV IgG titers correlated with these cells (ρ=0.6, p<0.0001).

*In vitro* stimulation of PBMCs with CMVpp65 and IL-2 or CMVpp65 alone, led to increased CD4+CD28null T cells in CMV+ HC with *in vivo* expansion of CD4+CD28null T cells. In CMV infected mice, there is a significant increase of CD4+CD28null T cells over time (day 8 vs. day 250 post infection, p<0.001).

The CMV infection related SNPs flanking MICB and TLR2, are not associated with CMV serology or CD4+CD28null T cells.

CD4+CD28null T cells were identified in mice and were significantly more present in EAE compared to control mice. Furthermore, CD4+CD28null T cells positively correlated with clinical score (0.42, p=0.008). CMV infected EAE mice had a worse disease course compared to EAE alone as indicated by their mean cumulative score (p<0.01). At day 30, the percentage of CD4+CD28null T cells was also higher in the CMV+EAE group compared to CMV or EAE alone, where expansions were also present. CD4+ T cell responses against MOG and CMV showed that there was no difference in CMV response between the different groups, but the MOG response did increase in the CMV+EAE group compared to EAE alone (p<0.01).

CONCLUSION

CMV status and IgG titers correlate with the percentage of CD4+CD28null T cells and CMV expands CD4+CD28null T cells in vitro and in vivo, indicating that CMV elicits expansion of CD4+CD28null T cells via repeated antigenic challenge.

CD4+CD28null T cells are increased in EAE mice and correlate with clinical score. CMV together with EAE lead to a higher EAE score and further increase in CD4+CD28null T cells compared to EAE alone. And CMV infection boosts the MOG specific CD4+ T cell responses. Thus CMV and CD4+CD28null T cells contribute to a worse EAE disease course.

Together these data suggests that CMV expands CD4+CD28null T cells and thereby aggravates MS disease. Further studies are warranted, but CMV vaccination to prevent CMV infection and these expansions might be beneficial for people at risk of developing MS.

REFERENCES